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ARMY MEDICAL BIOENGINEERING RESEARCH AND DEVELOPMENT --ETC F/G 6/5
CONCENTRATOR, MOSQUITO LARVAE.(U)
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USANBRDL-TR-7810

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Technical Report 7810

LEVEL II

Concentrator, Mosquito Larvae

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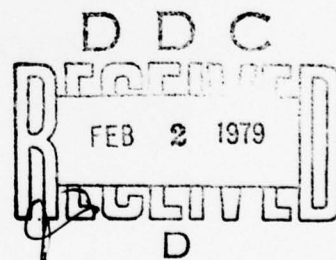
ADA064211

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FINAL TECHNICAL REPORT: August 1978

29 JAN 1979

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REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER TR-7810	2. GOVT ACCESSION NO.	3. RECIPIENT'S CATALOG NUMBER
4. TITLE (and Subtitle) Concentrator, Mosquito Larvae	5. TYPE OF REPORT & PERIOD COVERED Final Technical Report, FY75-78	
6. AUTHOR(s) Donald P. Driggers, CPT, MSC; H. Bruce Cranford, Ray E. Parsons, Ph.D., LTC, MSC; Robert E. Desrosiers, and James T. Kardatzke, Ph.D., CPT, MSC	7. PERFORMING ORG. REPORT NUMBER	
8. PERFORMING ORGANIZATION NAME AND ADDRESS Commander, U.S. Army Medical Bioengineering Research & Development Laboratory (USAMBRDL) ATTN: SGRD-UBH-P, Fort Detrick, Frederick, MD 21701	9. CONTRACT OR GRANT NUMBER(s)	
10. CONTROLLING OFFICE NAME AND ADDRESS US Army Medical Research & Development Command Fort Detrick, Frederick, Maryland 21701	11. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS 62.778A 3S762778A838.00.101	
12. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	13. REPORT DATE 24 Aug 78	
	14. NUMBER OF PAGES	
	15. SECURITY CLASS. (of this report) Unclassified	
16. DISTRIBUTION STATEMENT (of this Report) Approved for public release. Distribution unlimited.		
17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)		
18. SUPPLEMENTARY NOTES		
19. KEY WORDS (Continue on reverse side if necessary and identify by block number) Larval Concentrator Mosquito Habitats Mosquito Surveys Disease Vectors Population Studies		
20. ABSTRACT (Continue on reverse side if necessary and identify by block number) An improved portable immature mosquito concentrator system was developed and evaluated under laboratory and field conditions. In laboratory tests using 2500 <i>Culex pipiens</i> Linnaeus immatures, 99.8 percent of the specimens were captured by the concentrator system. When <i>Aedes taeniorhynchus</i> (Wiedmann) immatures were used, 93.7 percent of the specimens were captured. Excluding first instar larvae, the system caught 99.9 percent of the 5000 specimens used in the combined test. During field evaluation of the system, the contents of 100 dips made in a salt marsh habitat were concentrated into 10 collection and		

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storage vials. Of 6803 Aedes sollicitans (Walker) collected larvae, 100 percent of the specimens were captured by the improved immature mosquito concentrator system.

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SDS	Detl Section <input type="checkbox"/>
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I. INTRODUCTION

AUTHORITY:

Letter, SGRD-SDM, to USAMBRDL dated 31 March 1975, subject: Proposed Entomological Tasks.


BACKGROUND:

Adult and larval mosquito surveys are a basic requirement in comprehensive preventive medicine surveillance and control programs. Larval surveys are by necessity, time consuming as they involve actual search for breeding sites and physical collection of the specimens. Collection densities may range from zero to several hundred specimens per dip. Often, collection will be only 1 to 3 specimens per dip containing up to 300 ml of water. Since mosquito larvae are aquatic forms, these collections require the handling and processing of relatively large amounts of water. This is quite time consuming and cumbersome using standard methods.

During the late 1960's and early 1970's several articles were published reporting the existence of devices for concentrating collections of mosquito larvae. These articles and the relevant military need for such a device stimulated the U.S. Army Medical Bioengineering Research & Development Laboratory (USAMBRDL) to institute an in-house program to refine and develop a device for use during military pest management and surveillance operations.

PURPOSE:

To develop a mosquito larval concentrator for use in mosquito larva surveys for determination of population densities of potential disease vectors or pestiferous mosquitoes that affect the health and morale of military and associated populations in CONUS and at overseas locations.



II. METHODS AND MATERIALS

An immature mosquito concentrator system (Figure 1) was fabricated with modified commercially available materials and parts made from commercial standard stock items. The system consists of two basic components: the concentrator base unit (A-E) and the collection and storage vial assembly (F-I).

The primary component of the concentrator base unit is a standard polyvinylchloride (PVC) 3-inch to 1-1/2-inch reducing coupling (B), which serves as a receptacle funnel. A 3/4-inch diameter flat bottom hole 3/16-inch deep is drilled into the side of the funnel 3/4 inches from the top. This hole provides a flush fitting to attach the handle, a 6-inch long, 3/4-inch diameter PVC rod (C). The handle is fastened to the funnel with PVC pipe cement.

A bushing (D) is fabricated from a 1-inch section of 1-1/2-inch diameter, schedule 40 PVC pipe. The top is beveled at 48° to give a flush fit when the bushing is cemented into the base of the funnel with PVC pipe cement. A 3/16-inch deep, 1-46/64-inch diameter hole is bored into the bottom of the bushing to provide a seat for the thread insert (E). The thread insert is made by cutting the top out of the screw cap of a dollar-size coin storage tube. The thread portion is then cemented into the base of the bushing with a cyanoacrylate ester base glue.

A 3-9/16-inch diameter removable coarse screen (A) is cut from 4 x 4-mesh galvanized woven wire cloth. This is depressed into the large end of the funnel to form a snug friction fit.

Components of the collection and storage vial assembly are two 2-inch clear butyrate tube bodies (G) with screw caps (F) (commercially available silver dollar storage tubes), a 1-5/8-inch diameter collection screen (H) cut from 40 x 40 mesh monel wire cloth with 0.010-inch diameter wire and a 3/8-inch connector ring (I) cut from 1-3/4-inch clear butyrate tube stock. The two silver dollar coin storage tubes are cut to give 2-inch tube sections (G). These sections are assembled with the collection screen between them. This joint and the connector ring which encircles it are cemented with a cyanoacrylate ester base glue.

The immature mosquito concentrator system is utilized by removing both screw caps (F) from the collection and storage vial assembly and screwing either end into the bottom of the concentrator base unit. Contents of dips are poured into the funnel of the base unit. The water containing mosquito larvae and pupae passes through the coarse screen, which strains out large debris. It then passes through the 1-1/2-inch diameter hole in the base into the collection and storage vial.

Immature mosquitoes are trapped on the collection screen in the center of the vial assembly and the water passes out the bottom of the system.

Once collecting has been completed at a site or after a given number of dips have been concentrated, the cap is screwed onto the bottom of the vial and 20 to 100 ml of water is poured into the system to maintain the specimens concentrated in the container. The collection and storage vial assembly is then unscrewed from the concentrator base unit and capped. The unit may then be transported to another locale where the contents can be identified and analyzed.

Laboratory evaluation of the immature mosquito concentrator system was conducted using laboratory-reared mosquitoes in tap water. Twenty-five immature mosquitoes were introduced into beakers filled with either 100 or 250 ml of water. Contents were poured into the concentrator system and the effluent was collected in a white enamel pan and checked for specimens. Determinations were made of the number of specimens the collection system caught as well as the number it failed to catch. This test was replicated 10 times using Culex pipiens Linnaeus and Aedes taeniorhynchus (Wiedmann) mosquitoes. Pupae and larval instars were evaluated separately.

Field evaluation of the system was conducted in a salt marsh habitat near Wallops Island, Virginia. Two collectors and one observer participated in this evaluation. Using a 400 ml plastic dipper, each collector concentrated 10 dips into each of five separate collection and storage vials. While the collector was pouring the contents of the dipper through the concentrator system, the observer held a pail under the unit to catch the effluent so that any immature mosquitoes not captured on the wire cloth of the vial assembly could be collected and counted. The observer watched the collection vial and subjectively determined if the unit became clogged and to what degree. Clogging was rated on a scale of 0-5 with zero representing uninterrupted flow and five, total clogging.

Once field collections were complete, the collection and storage vials were taken to the laboratory where the mosquitoes were identified and counted, and their stage of development was determined. The number of specimens other than mosquitoes was also noted for each series of collections.

FINDINGS:

In laboratory evaluations using C. pipiens in 100 ml of water (Table 1), the system was successful in capturing 99.2 and 99.6 percent of first and second larval instars, respectively. One hundred percent of thirds, fourths and pupae were also caught. For C. pipiens in 250 ml water (Table 2), 99.6 percent of first instars were captured, while 100 percent of seconds, thirds, fourths and pupae were captured.

Table 1. Range and Mean Percent Culex pipiens Immatures in 100 ml of Water Captured in Ten Replicates by Improved Portable Immature Mosquito Concentrator System

Developmental Stage	Total Specimens		Percent Captured	
	Captured	Missed	Range	Mean
1st Instar	248	2	92-100	99.2
2nd Instar	249	1	96-100	99.6
3rd Instar	250	0	100-100	100.0
4th Instar	250	0	100-100	100.0
Pupa	250	0	100-100	100.0
Total	1247	3	92-100	99.8

Table 2. Range and Mean Percent Culex pipiens Immatures in 250 ml of Water Captured in Ten Replicates by Improved Portable Immature Mosquito Concentrator System

Developmental Stage	Total Specimens		Percent Captured	
	Captured	Missed	Range	Mean
1st Instar	249	1	96-100	99.6
2nd Instar	250	0	100-100	100.0
3rd Instar	250	0	100-100	100.0
4th Instar	250	0	100-100	100.0
Pupa	250	0	100-100	100.0
Total	1249	1	96-100	99.9

With immature Ae. taeniorhynchus in 100 ml of water (Table 3), 76.3 percent of first instars and 100 percent of seconds, thirds, fourths, and pupae were captured. For Ae. taeniorhynchus in 250 ml of water (Table 4), the concentrator system captured 62.0 and 98.8 percent of the first and second instars, respectively. One hundred percent of the thirds, fourths and pupae were captured.

Combined data (Table 5) for both C. pipiens and Ae. taeniorhynchus revealed catches of 96.8 percent of the 5000 immatures passed through the concentrator. Excluding first instars, the system captured 99.9 percent of specimens used in the combined test.

Earle (1956) reported losses by experienced collectors using the pipette and bottle system to be 23, 4 and 8 percent for early instars, late instars and pupae, respectively. When 2000 early instars, 2000 late instars and 1000 pupae were used to evaluate the concentrator system, losses were found to be 8.0, 0.0, and 0.0 percent, respectively. This represents a 2.88 fold increase over the pipette and bottle system of collecting and concentrating early instars from the dipper. If a collector is unwilling to accept this loss, a finer mesh screen may be used in the collection and storage vial assembly. However, a finer mesh increases the probability of clogging the system.

In the field evaluations (Table 6), the concentrator system captured 100 percent of the 6803 Ae. sollicitans larvae collected. First through fourth instars were present in the 100 samples dipped; no pupae were found. However, 88 other aquatic insects, primarily Hemiptera and Coleoptera, were also collected.

Although up to 2943 specimens were present in samples processed, clogging of the system was not found to be a problem in the field evaluation. Only two collections showed any clogging; both were minimal and neither interfered with field concentrating operations. Calculated mean clogging value in the field evaluation on the scale of 0-5 was 0.3.

Table 3. Range and Mean Percent *Aedes taeniorhynchus* Immatures in 100 ml of Water Captured in Ten Replicates by Improved Portable Immature Mosquito Concentrator System

Developmental Stage	Total Specimens		Percent Captured	
	Captured	Missed	Range	Mean
1st Instar	191	59	64-88	76.4
2nd Instar	250	0	100-100	100.0
3rd Instar	250	0	100-100	100.0
4th Instar	250	0	100-100	100.0
Pupa	<u>250</u>	<u>0</u>	<u>100-100</u>	<u>100.0</u>
Total	1191	59	64-100	95.3

Table 4. Range and Mean Percent *Aedes taeniorhynchus* Immatures in 250 ml of Water Captured in Ten Replicates by Improved Portable Immature Mosquito Concentrator System

Developmental Stage	Total Specimens		Percent Captured	
	Captured	Missed	Range	Mean
1st Instar	155	95	52-80	62.0
2nd Instar	247	3	96-100	98.8
3rd Instar	250	0	100-100	100.0
4th Instar	250	0	100-100	100.0
Pupa	<u>250</u>	<u>0</u>	<u>100-100</u>	<u>100.0</u>
Total	1152	98	52-100	92.2

Table 5. Combined Range and Mean Percent Aedes taeniorhynchus and Culex pipiens Immatures Captured in 40 Replicates by Improved Portable Immature Mosquito Concentrator System

Developmental Stage	Total Specimens		Percent Captured	
	Captured	Missed	Range	Mean
1st Instar	843	157	52-100	84.3
2nd Instar	996	4	96-100	99.6
3rd Instar	1000	0	100-100	100.0
4th Instar	1000	0	100-100	100.0
Pupa	1000	0	100-100	100.0
Total	4839	161	52-100	96.8

Table 6. Aedes sollicitans Larvae and Other Aquatic Specimens Collected During Field Evaluation by the Improved Portable Immature Mosquito Concentrator System

	Mosquito Larvae Instars				Other	Total
	1st	2nd	3rd	4th	Specimens	
Number Collected	1.	320.	815.	5667.	88	6891.
Percent of Total Collection	0.0	4.6	11.8	82.2	1.3	100.
Percent Missed	0.0	0.0	0.0	0.0	0.0	0.0
Percent Captured	100.	100.	100.	100.	100.	100.

III. DISCUSSION AND CONCLUSIONS

The concentrator system has proven to be a useful and reliable tool for use in mosquito larval surveys. Results obtained are reproducible and the system is superior to those previously described.

This system has broad application. It is easily portable and can be utilized by field units in any locality where mosquito larvae are present.

IV. RECOMMENDATIONS

That the Office of The Surgeon General (DASG-HCO) be advised that this concentrator system would be a suitable addition to the Entomological Collecting Kit, Field (NSN 6545-00-982-4121).

That this concentrator system be incorporated into the Entomological Collecting Kit, Field.

That a Research and Technology Work Unit Summary, DD Form 1498, be prepared reporting the completion of the work unit; Concentrator, mosquito larvae, 3S762778A838.00.101.

V. REFERENCES

Letter, SGRD-SDM to USAMBRDL dated 31 March 1975, subject:
Proposed Entomological Tasks.

Earle, H. H. 1956. Automatic device for the collection of aquatic
specimens. J. Econ. Ent. 49:261-262.

Fig. 1. Exploded diagram of improved portable immature mosquito concentrator system.

A-E Concentrator base unit;
A Coarse screen; B Funnel;
C Handle; D Bushing; E Thread
insert;

F-I Collection and storage
vial assembly; F Screw cap;
G Tube body; H Collection
screen; I Connector ring.

